# Role of Ghrelin Hormone in Systemic Lupus Erythematosus: Relation to Interferon alpha and Disease Activity Biomarkers.

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## Introduction

Systemic lupus erythematosus (SLE) is a severe multi-system autoimmune disease which results from both genetic predisposition and environmental factors **(Rahman, 2008).**

Ghrelin is a unique 28 amino acid peptide containing an n-octanoyl group on the serine in position 3 that was purified from rat stomach by Kojima et al, 1999. Ghrelin is the natural ligand for the growth hormone secretagogue (GHS) receptor (GHS-R) cloned in 1996 **(Howard et al, 1996)**. Circulating ghrelin consists of more than 90% of desacyl ghrelin and less than 10% acyl ghrelin **(Patterson et al, 2005)**.

In addition to the stomach (**Ariyasu et, 2001**), ghrelin is expressed in many tissues such as duodenum, jejunum, ileum, colon, lung, heart, pancreas, kidney, testis, pituitary, and hypothalamus **(Gnanapavan et al, 2002).**

One recent study evaluated ghrelin levels in pediatric patients with SLE (**Al et al, 2009**). However, it has not clarified whether ghrelin levels in SLE are related to the inflammatory process in such patients. Many other previous reports have focused on ghrelin role in cachexia of cardiovascular disease (**Leite-Moreira & Soares et al, 2007**) or chronic renal failure **(Mak et al. 2012).**

Interferon –α (INF-α) is an anti-viral cytokine in the type I INF family, which also includes INF-β, INF-ε, and INF-κ. Interferon alpha (IFN-α) and interferon beta (IFN-β), signal through the same type I interferon receptor, results in a wide range of effects upon the immune system. IFN-α normally functions in viral defense, and forms a bridge between the innate and adaptive immune systems (**Pestka et al, 2004**). In this way, IFN-α is also important in setting thresholds for self reactivity and autoimmunity. It was reported that some patients who have received recombinant human IFN-α injections to treat chronic viral infections or malignancy have developed de novo SLE (**Niewold, 2008**) which resolves with discontinuation of IFN-α therapy (**Niewold & Swedler, 2005**). Additionally, abnormally high levels of IFN-α are present in healthy first degree relatives of SLE patients as compared to healthy unrelated subjects (**Shahin et al, 2011**). Thus whether interferon-α is a causal agent in SLE or a secondary reactive finding in such disease remains to be further studied.

The primary aim of this study was to evaluate levels of ghrelin and INF-α in SLE patients and their relation to different clinical

manifestations of the disease. Moreover, to study their contribution to inflammatory process in patients with SLE, via estimating their correlation with disease activity markers in such patients.

**Subjects and Methods:**

Fifty four systemic lupus erythematosus patients (M/F: 10/44 , age 21.2 ± 11.1 years) (group I), were recruited in the study from patients attending Rheumatology and Immunology unit in Mansoura University Hospital, from the period of November 2012 to March 2013. Systemic lupus erythematosus patients meeting the revised American College of Rheumatology (ACR) classification criteria for SLE. Forty six age- and sex-matched healthy subjects served as control group (group II). Activity of SLE was evaluated according to systemic lupus erythematosus Disease Activity Index (SLEDAI).

All patients were subjected to full history taking and thorough physical examination. Body mass index (BMI) was calculated for all participants. Full immunological laboratory testing was performed to all patients including: ESR, CRP, C3, C4, ANA, and anti-dsDNA.

Total fasting plasma ghrelin hormone was measured for all the studied groups using enzyme linked immune sorbent assay (ELISA) kit according to Garcia et al (2005). Sun Red Human Ghrelin ELISA Kit, Catalogue No. 200-12-0973 was used in this study. Assay has a sensitivity of 0.6 pg/ml.

Serum level of IFN-which was measured using an enzyme linked immuno sorbent assay (ELISA) kit (Bender, Medsystems, Vienna, Austria). Aly et al, described a modified protocol to neutralize heterotrophile antibodies and avoid false-positive levels of IFN-a by adding 5% mouse serum to the assay buffer was used in the current assay **(Aly et al, 2004)**.

Inclusion criteria; all the studied patients fulfilled at least 4 of the 11 ACR classification criteria of systemic lupus erythematosus.

Exclusion criteria; patients with other autoimmune rheumatic diseases, type 1 diabetes mellitus, autoimmune thyroid disease, metabolic diseases, and pregnancy were excluded from the study. As INF-α has been implicated in the pathogenesis of these disorders to some degree.

Written informed consents were taken from all participants, and the study was approved by ethical committee of Mansoura Faculty of Medicine.

Immunosuppressive agents taken by enrolled patients were: hydroxy chloroquine (dose 200mg/day), azathioprine (100-150 mg/day), cyclophosphamide (1000 mg/month in pulse IV), and corticosteroids (dose from 10 to 40 mg/day). Details of treatment in each patient are described in the results.

## Results

This is case control study carried out on Fifty four systemic lupus erythematosus patients recruited in the study from patients attending Rheumatology and Immunology unit in Mansoura University Hospital **of mean age** 21.2± 11.1 years. In addition, 4**6** apparently healthy controls of mean age 22.9± 12.1 were included.

### ***Demographic and clinical data of the studied groups***

The patients and controls are well matched as regard age. There was no significant difference between patients and controls as regard weight, sex, DBP and SBP while there was significant difference as regard height & BMI. The frequency of mucocutaneous involvement among patient group was 81.5%, articular manifestations in about 74.1%, presence of lupus nephritis was confirmed in 64.8% while 25.9% of the patient group complained of presence of constitutional symptoms as shown in **table 1**.

### ***Laboratory findings in the studied groups***

As regard biochemical and hematological data between the patients group and the control group there was significant difference as regard HB, WBC, platelets, ESR, ANA titre, Anti ds DNA, INF α (**figure 1**) and gherlin hormone (**figure 2**) while there was no significant difference as regard HDL, LDL and triglycerides as shown in **table 2**.

### ***Correlation results***

#### ***1) Pearson correlation between gherlin hormone level and different study parameters***

Pearson correlation between gherlin hormone level and different study parameters showed significant correlation as regard weight (r=-0.3 & p=0.056), HDL (r=-0.3 & p=0.01), LDL (r=0.3 & p=0.03) (**figure 3**), SLEDAI (r=0.7 & p<0.0001), ANA (r=0.5 & p<0.0001), anti ds DNA (r=0.4 & p<0.002) and significant positive correlation with INFα (r=0.3 & p<0.01) (**figure 4)** as shown in **table 3**.Table

***2)Gghrelin hormone level in SLE patients according to the system afflicted.***

In our study we found significant increment in gherlin hormone level in SLE patients with lupus nephritis (**figure 5)** while there was no significant change in gherlin hormone level in SLE patients with mucocutaneous manifestations, articular involvement, Cerebritis, serositis, vasculitis and constitutional symptoms as shown in **table 4.**

Table (1) demographic data of studied groups.

| | Patients (n=54) | Control (n=46) | P |
|---|---|---|---|
| Age | 21.2± 11.1 | 22.9± 12.1 | ns |
| Female n(%) | 44 (81.5%) | 30 (65%) | ns |
| Height (m) | 1.6 ± 0.1 | 1.7 ± 0.1 | 0.003 |
| Weight (kg) | 71.8± 11.2 | 71.5± 7.4 | ns |
| BMI (kg/m2) | 27.1± 4.1 | 25.7± 1.3 | 0.03 |
| Systolic blood pressure | 129.1± 16 | 121.5± 8 | 0.004 |
| Diastolic blood pressure | 84± 12.4 | 78.3± 7 | 0.007 |
| Mucocutaneous | 44(81.5%) | - | - |
| Articular | 40 (74.1%) | - | - |
| Nephritis | 35 (64.8) | - | - |
| Constitutional | 14 (25.9%) | - | - |
| Serositis | 10 (18.5%) | - | - |
| Vasculitis | 8 (14.8%) | - | - |
| Cerebritis | 6 (11.1%) | - | - |
| Prednislone | 32±28mg/day | - | - |
| SLEDAI | 15±8.4 | - | - |

Table (2) laboratorial characteristics of SLE patients.

| | Patient group (n=54) | Control group (n=46) | P |
|---|---|---|---|
| Haemoglobin | 11.3 ± 1.9 | 13.5± 1.6 | <0.0001 |
| WBC | 4.7 ± 1.6 | 5.6± 0.9 | <0.0001 |
| Platelets | 162 ±107 | 377 ±118 | <0.0001 |
| ESR | 71.6 ±35 | 12.9± 2.6 | <0.0001 |
| ANA titre | 100.9± 51.1 | 5.8± 4.9 | <0.0001 |
| Anti ds DNA | 124.5± 49.3 | 11.2± 9.8 | <0.0001 |
| Cholesterol | 125.4 ±75.6 | 122.6 ±57.5 | ns |
| Triglycerides | 129.4± 57.9 | 115.3± 53.1 | ns |
| HDL | 40.7± 5.9 | 41.6 ±7.2 | ns |
| LDL | 179.2± 66.1 | 145.8±57.2 | 0.008 |
| INFα | 84.1± 56.5 | 5.1± 5.1 | <0.0001 |
| Ghrelin hormone | 429.1± 188.3 | 139.1± 62.3 | <0.0001 |

(3)Pearson's correlation between Ghrelin hormone and study parameters.

| | Patients (n=54) | | Control (n=46) | |
|---|---|---|---|---|
| | r | p | r | p |
| Age | -0.01 | ns | 0.1 | ns |
| Systolic blood pressure | -0.1 | ns | 0.2 | ns |
| Diastolic blood pressure | -0.2 | ns | 0.02 | ns |
| Height | -0.2 | ns | -0.2 | ns |
| Weight | -0.3 | 0.056 | -0.1 | ns |
| BMI | -0.2 | ns | 0.02 | ns |
| Cholesterol | 0.1 | ns | 0 | ns |
| Triglycerides | 0.03 | ns | 0.2 | ns |
| HDL | -0.3 | 0.01 | -0.1 | ns |
| LDL | 0.3 | 0.03 | -0.1 | ns |
| ESR | -0.1 | ns | 0.1 | ns |
| Hb | -0.04 | ns | -0.1 | ns |
| WBCs | 0.3 | ns | -0.1 | ns |
| Platelets | -0.1 | ns | 0.03 | Ns |
| SLEDAI | 0.7 | <0.0001 | - | - |
| ANA | 0.5 | <0.0001 | -0.1 | ns |
| Anti ds DNA | 0.4 | 0.002 | 0.1 | ns |
| INFα | 0.3 | 0.0 | -0.2 | ns |

| | | | | |
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Table (4)ghrelin hormone level in SLE patients according to the system afflicted.

| | Ghrelin hormone (mean±SD) | p |
|---|---|---|
| Mucocutaneous: | | |
| no (n=10) | 499.3± 249.8 | ns |
| yes (n=44) | 413.1± 171 | |
| Articular: | | |
| no (n=14) | 413±181.8 | ns |
| yes (n=40) | 475± 205.9 | |
| Nephritis: | | |
| no (n=19) | 360.3±130.8 | 0.004 |
| yes (n=35) | 466.4± 205.4 | |
| Cerebritis: | | |
| no (n=48) | 437.2± 197 | ns |
| yes (n=6) | 364.2±75.2 | |
| Serositis: | | |
| no (n=44) | 436.4± 174 | ns |
| yes (n=10) | 423.7±253. 4 | |
| Vasculitis: | | |
| no (n=46) | 417.8± 164.7 | ns |
| yes (n=8) | 494.3 ± 297.3 | |
| Constitutional: | | |
| no (n=40) | 411.9±171. 4 | ns |
| yes (n=14) | 479.8± 230 | |
| Haematologic: | | |
| no (n=34) | 436.2±183.6 | ns |
| yes (n=20) | 417.2± 200.3 | |

Figure 1:

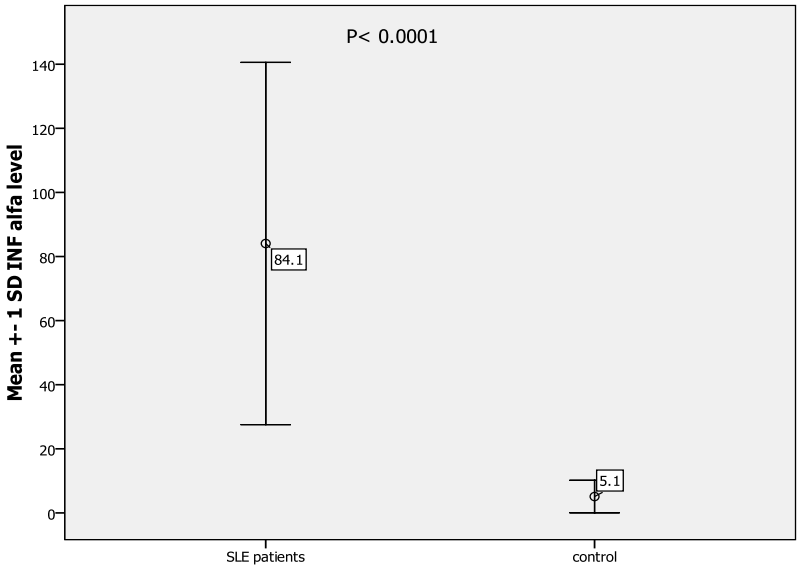


Figure 2:

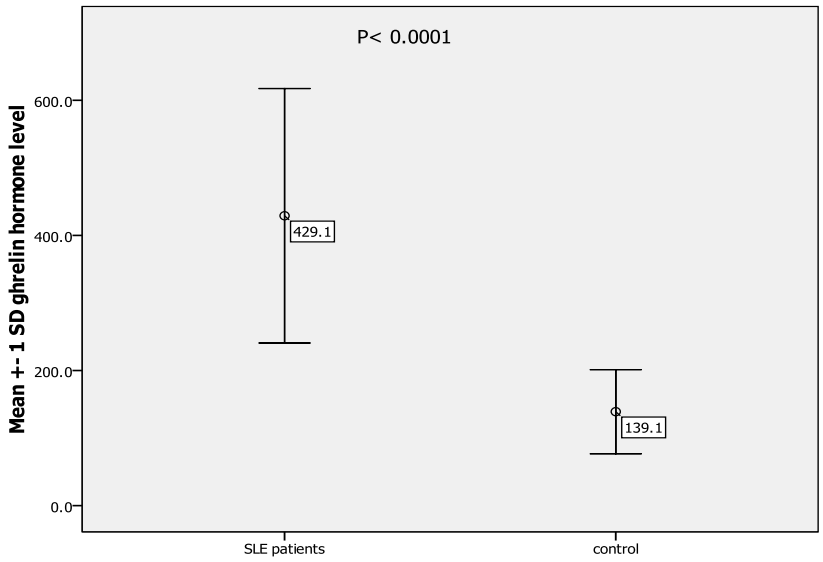



**Figure (3): linear regression analysis:** gherlin hormone level as an independent predictor for LDL level.

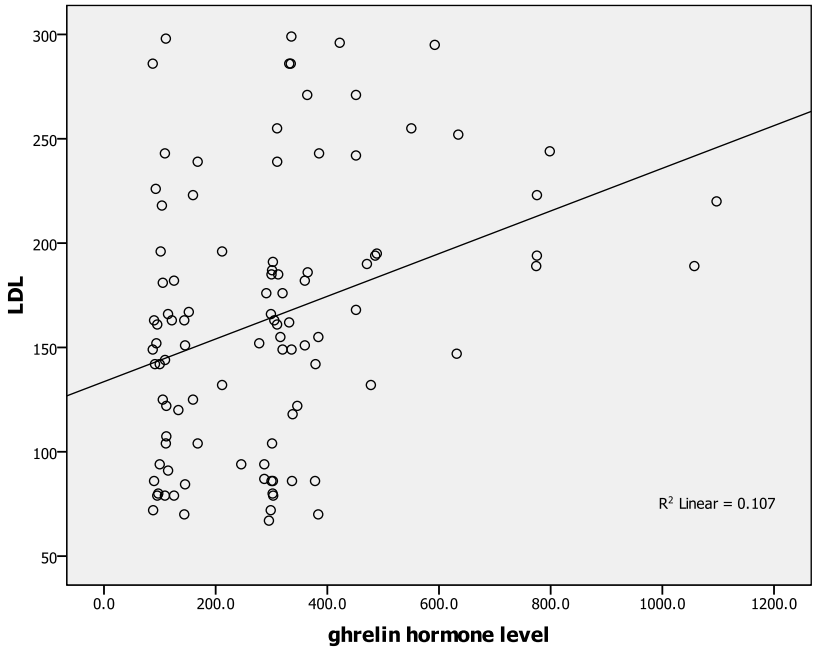


**Figure (4): linear regression analysis:** gherlin hormone level as an independent predictor for INFα level.

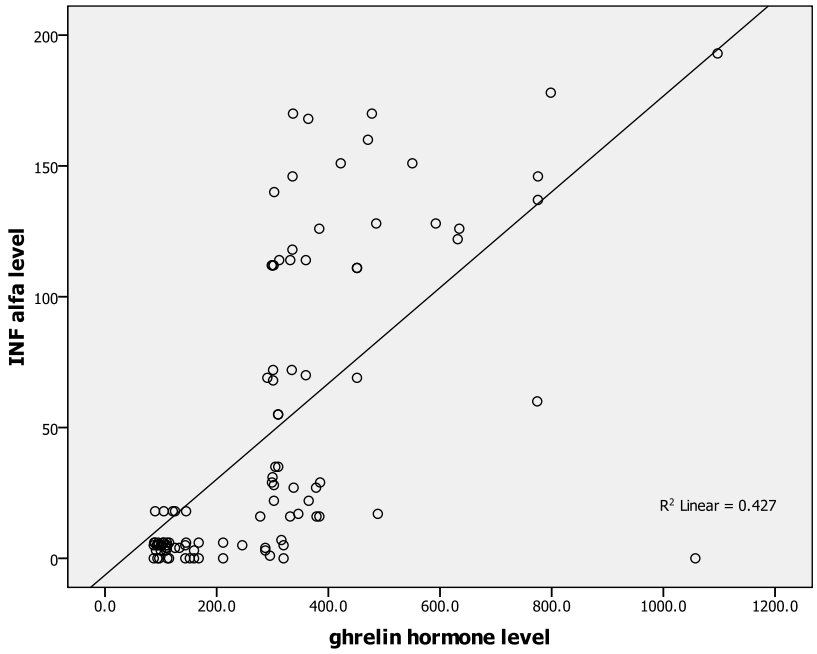
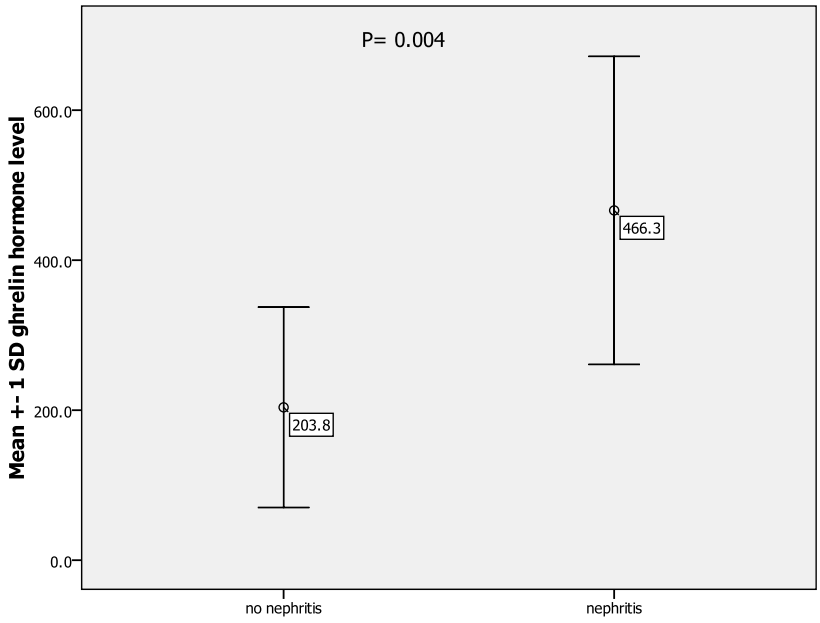



**Figure (5):** Gherlin hormone level in lupus nepritis patients.



## Discussion

Systemic lupus erythematosus (SLE) is a severe multisystem autoimmune disease which is caused by a combination of genetic and environmental factors **(Harley et al, 2006).** Many lines of evidence underscore the importance of cytokines in SLE susceptibility. Circulating interferon alpha (IFN-α) levels are high in many SLE patients **(Weckerle et al, 2011 & Ko et al, 2012)**. One of the most direct lines of evidence suggesting that high IFN-α is a primary pathogenic factor is that some individuals treated with recombinant interferon alpha (IFN-α) for viral hepatitis develop de novo SLE, which typically resolves when IFN-α treatment is discontinued **(Niewold and Swedler, 2005 & Niewold, 2008).** It has not yet been clarified whether gherlin hormone level in SLE is related to the inflammatory environment **(Kim et al, 2010).**

This is case control study carried out on fifty four systemic lupus erythematosus patients, were recruited in the study from patients attending Rheumatology and Immunology unit in Mansoura University Hospital, from the period of November 2012 to March 2013 and Forty six age- and sex-matched healthy subjects served as control group.

Both patients and control were subjected full history taking and thorough physical examination. Activity of SLE was evaluated according to systemic lupus erythematosus Disease Activity Index (SLEDAI). BMI was higher in SLE patients, this result was matched with Chaiamnuay et al, 2005 and Moc et al, 2008 which explained by glucocorticoid therapy, depression and more abnormal illness-related behaviors.

SBP and DBP were higher in SLE patients compared to control group these results were matched with Ryan, 2009 and Bourre et al, 2011 which explained by many mechanisms include the renin-angiotensin system, endothelin, oxidative stress, sex steroids, metabolic changes, peroxisome proliferator-activated receptor-gamma, and, perhaps most importantly, chronic inflammation and cytokines.

In our study we found pancytopenia in SLE patients compared to control group these results were matched with Tan et al, 1982, Hochberg, 1997, Ronnblom, 2010, Schur & Berliner, 2012 & Broder & Putterman,

2013. This can be explained by an immune mediated bone marrow failure, excessive peripheral cells destruction or certain drugs and infections.

As regard LDL & HDL there was significant increment in LDL and significant decrease in HDL among SLE patients, our results were in agreement with Gustafsson et al., 2009, Bengtsson et al., 2012 & Khaled and Naglaa, 2013 which explained by chronic inflammatory process and glucocorticoid therapy.

In agreement with Harley et al, 2006, Weckerle et al, 2011, Ko et al, 2012 and Dorothy et al., 2013 we found significant increment in IFN-alpha among SLE patient.

In our study we found significant higher level in gherlin hormone among SLE patient in contrary to KIM et al, 2010 who found lower gherlin hormone level in SLE patient this can explained by different patient inclusion criteria including age (34.6_6.7 years old), sex, steroid dosage (<7.5mg/day) and SLEDAI 3.24_2.81 (inactive disease) in comparison to our patients age (21.2± 11.1) and SLEDAI 15±8.4 (active disease).

We found significant positive correlation between gherlin hormone level and IFN-alpha level and other markers of lupus activity including ANA, anti ds DNA and SLEDAI while there was only significant positive correlation between gherlin hormone level and SLE patients with lupus nephritis these results may give an idea about the role of gherlin hormone in lupus activity in comparison with Kim et al, 2010 who found lower level of gherlin hormone among SLE patient with mild disease activity.

In conclusion these data suggest that possible role for gherlin hormone in the clinical manifestation of SLE patients especially those with lupus nephritis. Further longitudinal studies are required to evaluate the role that ghrelin plays in the assessment of disease activity of SLE.

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